Phase-shifting human circadian rhythms: influence of sleep timing, social contact and light exposure

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1. Both the timing of behavioural events (activity, sleep and social interactions) and the environmental light–dark cycle have been reported to contribute to entrainment of human circadian rhythms to the 24 h day. Yet, the relative contribution of those putative behavioural synchronizers to that of light exposure remains unclear.

2. To investigate this, we inverted the schedule of rest, sedentary activity and social contact of thirty-two young men either with or without exposure to bright light.

3. On this inverted schedule, the endogenous component of the core temperature rhythm of subjects who were exposed to bright light showed a significant phase shift, demonstrating that they were adapting to the new schedule. In contrast, the core temperature rhythm of subjects who were not exposed to bright light moved on average 0·2 h later per day and after 10 days had not significantly adapted to the new schedule.

4. The direction of phase shift in the groups exposed to bright light was dependent on the time of bright light exposure, while control subjects drifted to a later hour regardless of the timing of their schedule of sleep timing, social contact and meals.

5. These results support the concept that the light–dark cycle is the most important synchronizer of the human circadian system. They suggest that inversion of the sleep–wake, rest–activity and social contact cycles provides relatively minimal drive for resetting the human circadian pacemaker.

6. These data indicate that interventions designed to phase shift human circadian rhythms for adjustment to time zone changes or altered work schedules should focus on properly timed light exposure.

Circadian rhythms are biological oscillations with periods close to, but not exactly, 24 h. These rhythms are entrained or synchronized to the 24 h geophysical day by signals from the environment. The timing of the rest–activity schedule, social interactions, and exposure to light have all been hypothesized to entrain human circadian rhythms to the 24 h day (Wever, 1970; Aschoff, Fatranská, Giedke, Doerr, Stamm & Wisser, 1971; Klein & Wegmann, 1974; Wever, Polášek & Wildgruber, 1983; Lewy, Sack, Fredrickson, Reaves, Denney & Zielske, 1983; Daan & Lewy, 1984; Honma, Honma, Nakamura, Sasaki, Endo & Takahashi, 1995; Czeisler, 1995).

The role of light as a primary synchronizer of the human circadian pacemaker has been demonstrated in a variety of experiments (Wever *et al.* 1983; Dijk, Visscher, Bloem, Beersma & Daan, 1987; Lewy, Sack, Miller & Hoban, 1987; Honma, Honma & Wada, 1987; Czeisler *et al.* 1989; Czeisler, Johnson, Duffy, Brown, Ronda & Kronauer, 1990; Minors, Waterhouse & Wirz-Justice, 1991; Sack, Lewy, Blood, Keith & Nakagawa, 1992; Van Cauter *et al.* 1993; Czeisler, 1995). A neuroanatomical basis for this effect of light has been documented (Morin, 1994). Photic information responsible for synchronization of the circadian system to the environmental light–dark cycle is transmitted from the retina to the circadian pacemaker, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, via the retinohypothalamic tract (RHT) (Moore & Lenn, 1972; Friedman, Johnson, Chorsky & Stopa, 1991).

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Unfortunately, quantification of the extent to which light *alone* is responsible for inducing phase shifts (changes in the timing of circadian rhythms) in humans has proved more difficult than in other species. Detailed studies of the phase-shifting effects of light in mammals have been carried out largely in species without consolidated sleep episodes. In mammals, as in most other species studied, light exposure in the early subjective night causes phase delay shifts (to a later hour), light in the late subjective night causes phase advance shifts (to an earlier hour), and light during the subjective day causes modest changes in phase. The most sensitive time, i.e. the time when the largest responses are seen, is during the subjective night (Daan & Pittendrigh, 1976). While exposure of mammals to light during the subjective night can sometimes induce acute changes in activity directly associated with the light exposure, nocturnal mammals have their major bout of activity during the subjective night and therefore do not generally require a change of their rest–activity cycle to expose them to light at the most sensitive times of the circadian cycle. Most of the diurnal mammals studied to date (with the exception of the squirrel monkey – Hoban & Sulzman, 1985) have polyphasic rather than consolidated sleep episodes. Therefore, even when given a light pulse during the subjective night (while relatively inactive) diurnal mammals without consolidated sleep episodes are likely to be awake a substantial portion of the time so that light is able to reach the SCN via the RHT without attenuation by eyelid closure.

In contrast, exposure of humans to light during the time of peak photic sensitivity, the subjective night (Czeisler *et al.* 1989), is complicated, since humans are generally asleep with their eyes closed at night. One experimental approach has been to shift acutely the rest–activity cycle so that subjects are awake at night in order to ensure exposure to the light stimulus. However, this complicates interpretation of the results, since in some mammals activity, feeding and social interaction have been reported to entrain and/or induce phase shifts (Mrosovsky, Reebs, Honrado & Salmon, 1989; Van Reeth & Turek, 1989; Boulos, Frim, Dewey & Moore-Ede, 1989; Edgar & Dement, 1991; Gannon & Rea, 1995; Goel & Lee, 1995). Changing the timing of the rest–activity schedule confounds interpretation of observed light-induced phase shifts in humans. These might have been influenced by the altered rest–activity, social contact, sleep and meal schedule inherent in such protocols (Czeisler *et al.* 1989, 1990; Eastman, 1992; Boivin, Duffy, Kronauer & Czeisler, 1996).

Therefore, the present study was designed to investigate the possible contribution of alterations in the timing of behavioural events (sedentary activity, sleep, social contact and food intake) on phase shifts elicited by nocturnal light exposure in humans. To do this, we inverted the rest–activity, sleep–wake, social contact and food intake schedule while exposing half of the subjects to a bright light stimulus and half to darkness in a randomized dim light trial.

METHODS

Subjects

Thirty-two young men (mean age ± S.D., 21·7 ± 0·6 years) were studied. Subjects were free from medical, psychiatric and sleep disorders as determined by history, physical examination, biochemical screening tests, chest radiographs, electrocardiograms, and psychological screening questionnaires (the Minnesota Multiphasic Personality Inventory and the Beck Inventory). Subjects were instructed to abstain from caffeine, nicotine, alcohol, and drugs (including all prescription and non-prescription medications) for the 3 weeks before their study; their compliance was verified on the day of admission to the laboratory with urinary toxicological analysis, which included testing for caffeine, nicotine and common drugs of abuse.

All subjects denied a history of night work or shift work in the 3 years prior to study, and none reported crossing more than two time zones in the 3 months prior to study. Subjects were instructed to keep a regular sleep–wake schedule (bedtimes and waketimes within 1 h of self-selected target times) during the 3 weeks prior to their admission to the laboratory. Adherence to a regular schedule during the week immediately prior to admission was verified with a wrist actigraph (Vitalog Monitoring, Inc., Redwood City, CA, USA); only subjects who maintained the regular schedule as instructed were admitted to the laboratory for the study.

Each subject underwent an endogenous circadian phase and amplitude assessment following three baseline nights in the laboratory (Brown & Czeisler, 1992). To minimize uncertainty in the phase estimation procedure, only those subjects with an initial endogenous temperature amplitude which exceeded 0·14 °C were empanelled into the study (Czeisler *et al.* 1989). In addition, only those subjects whose initial phase of the fitted minimum of the endogenous temperature cycle fell within normal limits for healthy young men, defined as the population mean ± 3 S.D. (02.22 h to 10.23 h (Czeisler *et al.* 1992)), were empanelled into the study. Each of the thirty-two subjects was randomly assigned into one of four study groups. All thirty-two subjects completed the first segment of the protocol, involving assessment of their response to a three-cycle stimulus; twenty-nine subjects completed the second segment of the protocol, measuring their response to an additional three cycles of the stimulus.

All protocols used in the study were reviewed and approved by the Human Research Committee of the Brigham and Women's Hospital. Each subject gave signed informed consent prior to beginning the laboratory portion of his study.

Protocol

Each 15 day study began with three baseline days and nights in the laboratory (see Fig. 1), with subjects scheduled to sleep for 8 h at their habitual times (the average of the 7 self-reported bedtimes and waketimes from the week prior to admission). After these three adaptation nights, each subject began a constant routine (CR) to assess the endogenous circadian phase and amplitude of his core temperature rhythm (CR 1). The constant routine procedure was designed to eliminate or distribute evenly across the circadian cycle factors known to mask the endogenous rhythm of core temperature. Therefore, subjects, as described in detail elsewhere (Czeisler *et al.* 1990), were kept awake in a constant semi-recumbent posture in dim light (10–15 lux) with minimal physical activity allowed, while nutrition was distributed in hourly snacks across the 24 h day.

Circadian phase was defined as the minimum of the core body temperature cycle – the estimated circadian phase of the

temperature minimum (ECP_{min}) – measured during the CR (see below). In order to standardize the protocol across subjects, we used each subject's circadian phase from his first CR to schedule all subsequent protocol events (see Table 1). In order to schedule the midpoint of the subsequent rest episode 12 h opposite to the midpoint of the wake episode, the initial CR was truncated after 26–33 h, shorter than the usual 40 h length (Brown & Czeisler, 1992).

After an 8 h recovery sleep episode, subjects underwent 3 days on an inverted rest–activity schedule, consisting of 16 h of wakefulness in dim light (10–15 lux) per day and a scheduled 8 h bedrest episode in darkness. During the waking episode, subjects were exposed to a 5 h episode of either bright light (7000–13 000 lux) or darkness (<0·03 lux) which was centred in the middle of the waking day.

In Groups 1 and 2, the timing of the 5 h stimulus was such that the stimulus was centred at a phase at which bright light would be predicted (Czeisler *et al.* 1989; Klerman, Dijk, Kronauer & Czeisler, 1996) to produce a phase delay of the circadian oscillator ('delay schedule'), while in Groups 3 and 4 the timing of the stimulus was such that bright light would be predicted to produce a phase advance of the oscillator ('advance schedule'). The timing of the stimuli for both schedules was chosen such that the predicted phase advance and phase delay shifts to bright light would be of equal magnitude (Klerman *et al.* 1996). This resulted in an average delay in the timing of the bedrest episode of 12·2 h for the delay schedule

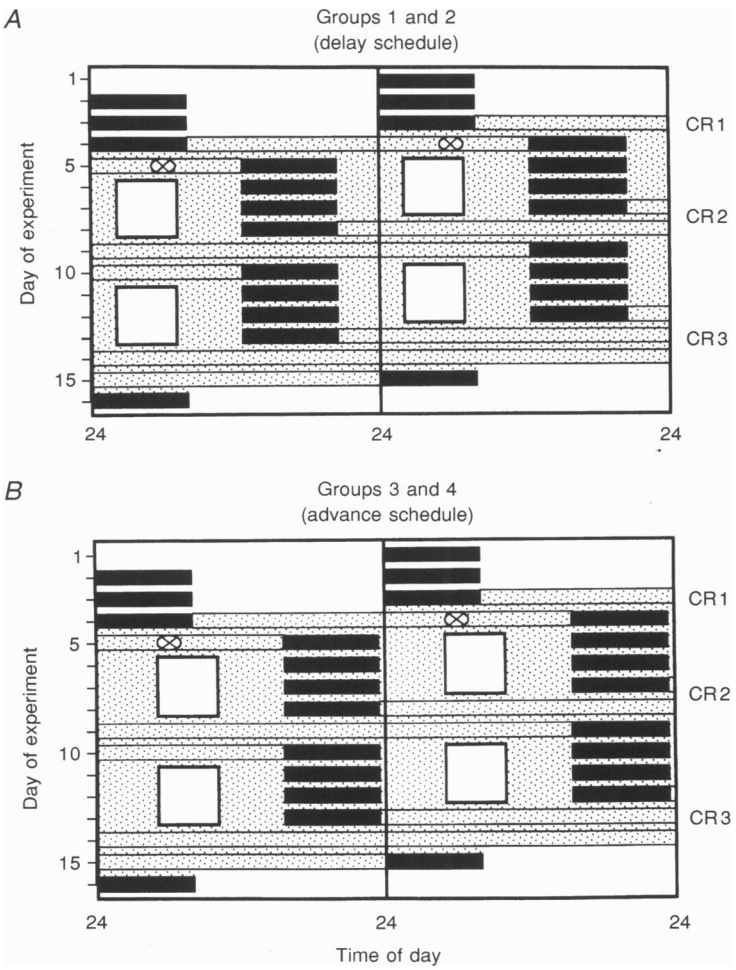

Figure 1. Double raster plots of study protocols

Time of day is plotted on the x-axis and successive days of the experiment are plotted both beside and underneath each other. Filled bars represent scheduled bedrest episodes, stippled bars represent constant routine (CR) procedures, stippled area represents when ambient lighting was 10–15 lux, open boxes represent 5 h per day stimuli (bright light or darkness), ⊗ represent the estimated circadian phase of the temperature minimum (ECP_{min}) during the first constant routine (CR 1). *A*, protocol for groups 1 (darkness) and 2 (bright light). The centre of both the wake episode and the 5 h stimulus was scheduled 22·5 h after the ECP_{min} as measured on CR 1. The centre of the 8 h bedrest episode was scheduled 12 h opposite the centre of the wake episode. This resulted in the rest–activity schedule being shifted an average of 12·2 h later than the subjects' habitual times. *B*, protocol for groups 3 (darkness) and 4 (bright light). The centre of both the wake episode and the 5 h stimulus was scheduled 1·5 h after the ECP_{min} as measured on CR 1. The centre of the 8 h bedrest episode was scheduled 12 h opposite the centre of the wake episode. This resulted in an average shift of the rest–activity schedule to 8·4 h earlier than the habitual times.

Table 1. Protocols used in the study

Group	Stimulus			Bedrest episode			
	Centre	Begin	End	Centre	Begin	End	Change
Delay schedule							
1. Dark 2. Light	22.50	20.00	01.00	10.50	06.50	14.50	−12·2 h
Advance schedule							
3. Dark 4. Light	01.50	23.00	04.00	13.50	09.50	17.50	+8·4 h

All times shown are with reference to the minimum of the core temperature cycle (the estimated circadian phase of the temperature minimum, ECP_{min}) as measured during the first constant routine (CR 1). The centre of the wake episode was scheduled to be 12 h opposite the centre of the 8 h bedrest episode, with the 5 h stimulus centred within the wake episode. The average number of hours the bedrest episode was shifted from baseline is shown in the right column.

groups and an advance of 8·4 h for the advance schedule groups (see Table 1). Partial data from the subjects in Groups 3 and 4 (data from the first two CRs) were incorporated into the construction of a dose–response curve for the resetting effects of light, which was published recently (Boivin *et al.* 1996).

After three cycles on this schedule, a second constant routine (CR 2) was carried out for 40 h. Following another 8 h recovery sleep episode, the three-cycle schedule was repeated at the same clock hours. A third constant routine (CR 3) was carried out for 40–50 h, and this was followed by a final recovery sleep episode of at least 8 h, after which time the subject was discharged from the laboratory.

Light or darkness exposure

The 5 h bright light exposures were provided by ceiling-mounted cool-white fluorescent lamps (North American Philips Lighting Corp., Bloomfield, NJ, USA). Prior to the beginning of the bright light exposure, light levels were increased from ambient room illumination (10–15 lux) to ~10 000 lux in six linearly graded steps lasting 5 min each. At the end of the 5 h episode, six 5 min steps were used to return the room to 10–15 lux. Ocular light exposure was estimated three times per hour during the 5 h bright light exposure from a sensor held at the level of the subjects' forehead and pointed in the direction of gaze (International Light, Newburyport, MA, USA). Subjects were instructed to gaze at a spot on the wall from which they would be exposed to > 9000 lux of light for 10 min out of each 20 min during the 5 h bright light episode. During the other 10 min of each 20 min segment, subjects were allowed to read or look down.

Darkness exposure was achieved by switching off all lights in the windowless room of the subject. A technician remained in the room throughout the 5 h darkness episode to engage the subject in conversation and verify wakefulness. Ambient light intensity measurements during dark episodes were taken from a sensor in the room, connected to a meter outside the room through an insulated porthole in the wall.

All subjects wore clear ultraviolet-excluding safety glasses (Uvex Winter Optical, Inc., Smithfield, RI, USA) throughout each 5 h exposure to light or darkness.

Temporal isolation

Throughout the 15 day study, each subject lived alone in a sound-attenuated room isolated from time cues. To achieve this, the room had no clock, window, television, telephone or radio, and neither subject nor staff members wore a watch. Furthermore, subjects could not have visitors or receive telephone calls during their study. Subjects were allowed to perform their usual daily activities within their room, and could receive mail, watch videos and listen to music, but were restricted from exercise during their study.

Social contact

During scheduled wake episodes, technicians entered the subject's room several times per hour to administer subjective alertness and/or performance tests (see below). In addition, technicians entered the subject's room to wake him, serve three meals and a snack each day, provide a urinal upon request, place facial and scalp electrodes prior to each sleep recording, and prepare for the bright light or darkness exposure.

During the scheduled darkness/sleep episodes, technicians only entered a subject's room briefly (< 5 min) if he requested a urinal. If a subject was unable to remain asleep during a scheduled sleep episode, he was instructed to remain lying in bed in the dark until a technician came to 'wake' him.

Data collection

Throughout the study, core temperature was collected at 1 min intervals from a rectal thermistor (Yellow Springs Instrument Company, Yellow Springs, OH, USA).

Sleep was polygraphically recorded during each scheduled sleep episode and during the constant routines. During waking episodes and throughout each constant routine, subjective alertness was assessed several times per hour using a non-numeric visual analog scale and calculation performance tests were administered hourly. During certain portions of the study, blood samples were collected several times per hour from an indwelling intravenous catheter inserted in a forearm vein. Samples were frozen at −20 °C for later hormonal analysis. These data will be reported elsewhere.

Data analysis

Phase and amplitude of core temperature data were estimated by fitting a two-harmonic-regression model with first-order auto-

regressive noise to the data from the constant routines (Brown & Czeisler, 1992). The minimum of the fitted model (referred to as the estimated circadian phase of the temperature minimum, ECP_{min}) from the initial constant routine was used as a phase reference marker for determining the timing of the remainder of the protocol and for comparing the results of the analysis from the second and third constant routines in order to calculate changes resulting from the intervention. Amplitude was defined as half the distance between the maximum and minimum of the first harmonic component of the model.

The SAS system (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Analysis of variance (ANOVA) was used to compare the four groups at baseline. Comparisons between control and light treatment groups of subjects on the advance and delay schedules were made using Student's t test. Comparisons of phase shifts between groups of subjects were made using ANOVA for repeated measures. All results are presented as means ± 1 S.E.M. unless otherwise indicated.

RESULTS

The minimum of the core temperature cycle (ECP_{min}) during the first constant routine (CR 1) occurred on average at 06.24 ± 0.14 h ($n = 32$), a mean of 2·15 h before the subjects' habitual waketime of 08.33 ± 0.08 h ($n = 32$). The mean amplitude of the core temperature rhythm was 0·29 ± 0·02 °C ($n = 32$). Analysis of variance detected no significant differences between the four groups in their average age, habitual bedtime or waketime, ECP_{min} or temperature amplitude on CR 1.

Delay groups

After the first three-cycle stimulus, the ECP_{min} of subjects in group 1 (delay schedule, darkness) drifted an average of 0·26 ± 0·48 h later between CR 1 and the second constant routine (CR 2) to 06.56 h ($n = 8$). In contrast, the ECP_{min} of seven of the eight delay subjects in group 2 (delay schedule, bright light) shifted 6·91 ± 0·49 h later to 12.24 h ($n = 7$), a significantly greater shift than that observed in those subjects exposed to darkness ($P < 0·00001$). However, one subject (1118) in group 2 phase-advanced by 5·6 h and his data were excluded from subsequent analysis (see below). There was no significant change in amplitude from CR 1 to CR 2 in either group.

When the same three-cycle stimulus was repeated, subjects who received light continued to delay further than those who did not: their ECP_{min} on the third constant routine (CR 3) was 14.48 ± 1.00 h ($n = 7$) *vs.* 09.32 ± 0.50 h ($n = 6$; one of the control subjects withdrew from the study before CR 3). This resulted in a total delay shift between the first and third CR of 09·32 ± 0·55 h ($n = 7$) for the group who received bright light exposure while the group who did not shifted 3·24 ± 1·11 h ($n = 6$; $P < 0·0003$; see Fig. 2).

Advance groups

After the first three-cycle stimulus, the ECP_{min} of subjects in group 3 (advance schedule, darkness) drifted a mean of 1·05 ± 0·38 h later between CR 1 and CR 2, to 07.38 ± 0.19 h ($n = 8$). In contrast, the ECP_{min} of subjects in group 4 (advance schedule, bright light) shifted 4·75 ± 0·41 h earlier between CR 1 and CR 2, to 01.54 ± 0.33 h ($n = 8$; $P < 0·0001$).

When the same three-cycle stimulus was repeated, the phase shifts seen were again significantly different. Subjects in group 3 (darkness) drifted a mean of 0·69 ± 0·44 h later to 08.20 ± 0.25 h ($n = 8$) while those in group 4 (bright light) shifted 2·85 ± 0·31 h earlier, to 23.22 ± 0.45 h ($n = 7$; one subject withdrew before CR 3; $P < 0·0001$). Overall, between the first and third CR, the subjects who received bright light phase advanced by 7·38 ± 0·44 h ($n = 7$), while those who did not receive light drifted later by 1·74 ± 0·63 h ($n = 8$; $P < 0·0001$; see Fig. 2). The time of the ECP_{min} on CR 3 differed by nearly 9 h depending on the treatment, with subjects who received bright light exposure during the two three-cycle stimuli shifting their ECP_{min} earlier to a mean of 23.22 h, while the ECP_{min} of subjects exposed to darkness during the two three-cycle stimuli drifted later to a mean of 08.20 h.

Delay *vs.* advance groups

Within-group phase shift results for the two groups exposed to bright light were consistent, with one exception. The ECP_{min} of subject 1118 (delay schedule, light) phase advanced rather than phase delayed. The stimulus in these studies was designed to be centred only 1·5 h from the crossover point in the phase response curve between

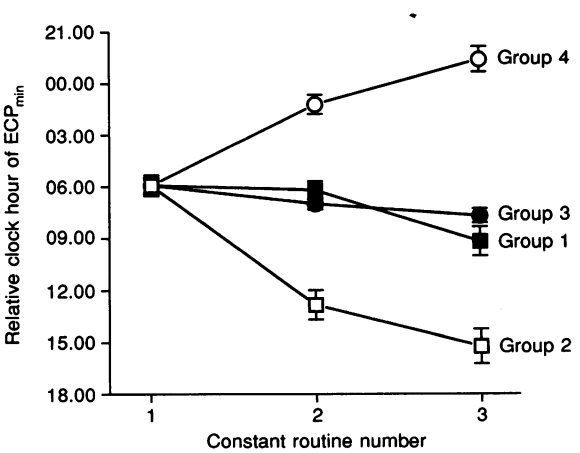

Figure 2. ECP_{min} on CR 1, 2 and 3

Mean (± S.E.M.) time of the minimum of the core temperature cycle (ECP_{min}) for the four groups is plotted for each of the constant routine phase assessments. Data for each group are plotted with respect to ECP_{min} on CR 1, with ECP_{min} on CR 1 = 06.00 h. Squares, groups 1 and 2, delay schedule; circles, groups 3 and 4, advance schedule; filled symbols, control groups; open symbols, light groups.

advances and delays (Czeisler *et al.* 1989). We presume that the phase of this subject was misestimated during the first shortened CR required for this protocol. The phase estimation procedure has a standard deviation of $\pm 0{\cdot}9$ h (Kronauer, Jewett & Czeisler, 1993), so that an error in the initial phase estimate of only $1{\cdot}7\,\sigma$ (i.e. 1·5 h) could result in the application of the bright light just after rather than just before the ECP_{min} (leading to an advance, rather than a delay shift).

After the initial three-cycle intervention stimulus, the *magnitude* of the phase shift in the two light groups appeared to be significantly different (advance group, $+4{\cdot}75 \pm 0{\cdot}41$ h; delay group, $-6{\cdot}91 \pm 0{\cdot}49$ h; $P < 0{\cdot}01$; see Fig. 3*A*). However, when normalized to account for the expected drift of phase due to the slightly longer than 24 h intrinsic period of the human circadian pacemaker (Czeisler *et al.* 1995*a*), no significant difference remained in the magnitude of the phase shift between the advance schedule light group and the delay schedule light group (5·75 *vs.* 6·11 h; $P = 0{\cdot}5807$; see Fig. 3*B*), although the direction of phase shift was opposite.

Control groups

When the ECP_{min} of the control subjects in groups 1 and 3 were compared, there was no significant difference between them on CR 1, 2 or 3 (groups 1 *vs.* 3: CR 1, 06.40 ± 0.36 *vs.* 06.36 ± 0.27 h, $P > 0{\cdot}9$; CR 2, 06.56 ± 0.30 h *vs.* 07.38 ± 0.19 h, $P > 0{\cdot}2$; CR 3, 09.32 ± 0.50 h *vs.* 08.20 ± 0.25 h, $P > 0{\cdot}1$). A two-factor ANOVA for repeated measures with factors CR number (1, 2 or 3) and group (advance schedule or delay schedule) revealed a significant effect of CR number, indicating that there was an overall drift of phase to a later hour between CR 1 and CR 3 ($F_{2,24} = 15{\cdot}28$, $P < 0{\cdot}001$). Neither the factor group nor the interaction between CR number and group was significant, indicating that there was

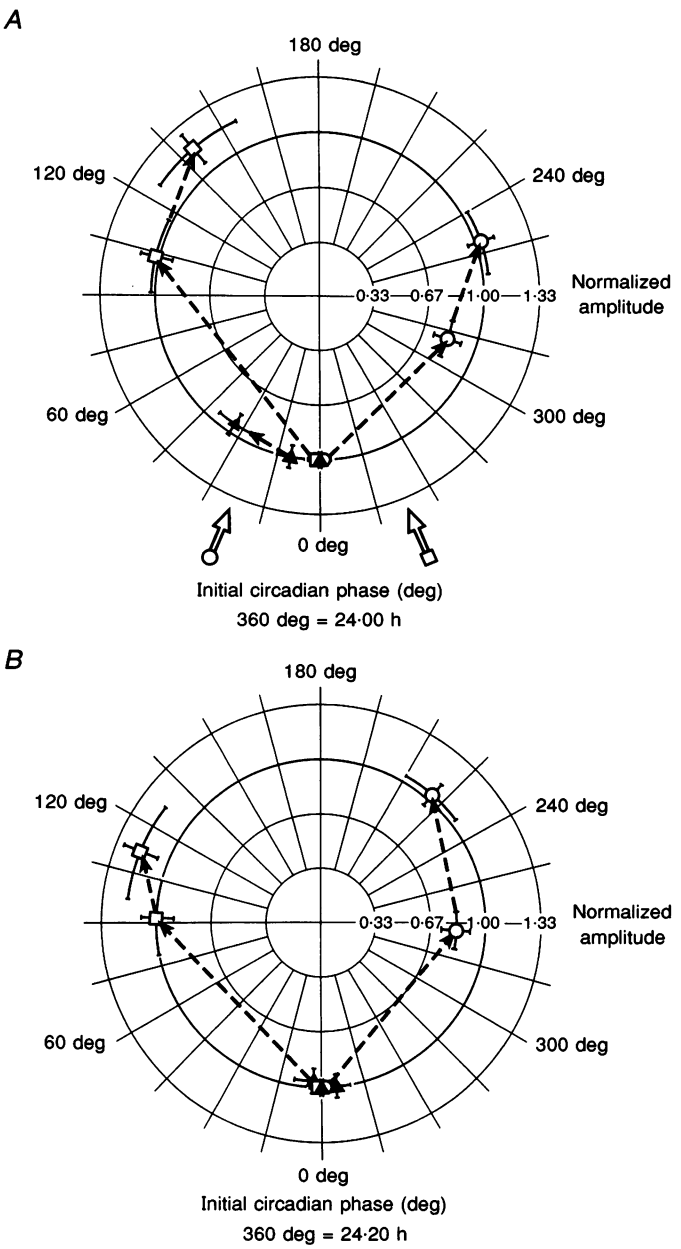

Figure 3. Polar representation of phase and amplitude on CR 1, 2 and 3

Circadian phase is represented in degrees, with ECP_{min} on CR 1 normalized to 0 deg. Amplitude is indicated on the radius, with temperature amplitude on CR 1 normalized to a value of 1·00. Data are means ± S.E.M. Time proceeds in a clockwise direction, so phase delay shifts are plotted in a clockwise direction and phase advance shifts are plotted counterclockwise. Dashed lines indicate the change in relative phase and amplitude in subsequent CRs. □, group 2; ○, group 4; ▲, groups 1 and 3. *A*, open arrows indicate the relative time of the centre of the waking episode with respect to the ECP_{min} on CR 1 for groups 2 (□) and 4 (○). 360 deg is equal to 24·00 h. *B*, 360 deg is equal to 24·20 h. This representation takes into account the drift of phase due to the slightly longer than 24 h intrinsic period of the human circadian system when studied under these laboratory conditions.

no significant difference in the amount of drift between the two control groups ($P > 0{\cdot}1$).

When normalized to account for intrinsic circadian period, the average phase for groups 1 and 3 (darkness/control) was not significantly different over the course of the study, despite 10 days on an inverted sleep–wake schedule ($F_{2,24} = 1{\cdot}02$, $P = 0{\cdot}36$; see Fig. 3*B*).

DISCUSSION

The present study indicates that the timing of periodic behavioural stimuli, such as sleep, sedentary activity, social contacts and food intake are relatively weak circadian synchronizers in humans and provides further evidence that exposure to light is the primary synchronizer of the human circadian pacemaker. The endogenous core temperature rhythm of control subjects in the present study failed to adjust significantly toward the shifted rest–activity schedule. In fact, after 10 days on an inverted schedule, including inversion of the timing of social contact, bedrest, sedentary activity and meals, the ECP_{min} of fifteen of the sixteen control subjects was still occurring during the scheduled activity period. These subjects showed only small changes in phase to a later hour, suggesting that they were drifting at a rate established by the intrinsic period of their circadian system (Czeisler *et al.* 1989; Campbell, Dawson & Zulley, 1993; Czeisler *et al.* 1995*a*) rather than adjusting to the inverted schedule.

In contrast to the results from the control groups, subjects in both groups who were exposed to the bright light stimulus showed robust shifts in phase. In all cases, after 10 days on an inverted rest–activity schedule with bright light exposure during six of the daily activity periods, the ECP_{min} was shifted such that it occurred during or within an hour of the imposed darkness/sleep episode. The magnitude and direction of the phase shifts observed in the two light groups was consistent with our earlier studies of the effect of light on the human circadian system (Czeisler *et al.* 1989, 1990) and with that predicted by Kronauer's mathematical model of the effect of light on the human circadian system (Kronauer & Czeisler, 1993), although the average response of the phase advance group was somewhat more modest than that predicted by the model (Klerman *et al.* 1996). Given that phase advance shifts are often associated with transients (Pittendrigh & Daan, 1976), further studies will be required to determine whether this difference between the model-predicted and the observed phase advances can be attributed to transients.

The finding that control subjects failed to adjust to an imposed schedule, even after 10 days on that schedule, is consistent with results from a number of studies in the blind. In many cases of total blindness, regular schedules of activity, work, meals, rest and social contact fail to reset the circadian pacemaker by the few minutes required to entrain the pacemaker's slightly longer than 24 h period to the 24 h day (Miles, Raynal & Wilson, 1977; Orth, Besser, King & Nicholson, 1979; Sack *et al.* 1992; Klein, Martens, Dijk, Kronauer, Seely & Czeisler, 1993; Czeisler *et al.* 1995*b*). However, it has been reported that some bilaterally enucleated humans appear to be entrained by such behavioural stimuli (Sack *et al.* 1992; Czeisler *et al.* 1995*b*), suggesting that non-photic synchronizers too weak for detection by the present protocol may be sufficient for entrainment in some cases. This would be consistent with data from both animals (Van Reeth & Turek, 1989; Mrosovsky *et al.* 1989; Edgar & Dement, 1991; Gannon & Rea, 1995) and humans (Van Reeth *et al.* 1994) indicating that vigorous activity/exercise can act as a circadian synchronizer. In fact, the timing of the imposed rest–activity schedule may explain why one control subject in the present study did appear to adjust to the imposed rest–activity schedule after 10 days. Further studies are needed to explore whether vigorous exercise, in contrast to the sedentary activity of subjects in the present study, can entrain the human circadian pacemaker to the 24 h day.

Our findings initially appear to be inconsistent with the recent results of Honma *et al.* (1995), who report significant phase advances in control subjects on a rest–activity schedule similar to that of our advanced schedule groups. However, control subjects in their study were exposed to 300–500 lux of light throughout their scheduled wake episodes, while control subjects in the present study were in 10–15 lux of light or darkness. Given our more recent data on the effectiveness of ordinary room light (~180 lux) in shifting human circadian rhythms (Boivin *et al.* 1996), together with simulations based on the mathematical model of Kronauer (Klerman *et al.* 1996) we would expect subjects exposed to 300–500 lux each night for five to ten nights to exhibit significant phase shifts due to the ambient light exposure alone. We conclude that our present data are therefore not inconsistent with those of Honma *et al.* Taken together, they illustrate the powerful effect of light as the primary synchronizer of the human circadian pacemaker and support the conclusion that the resetting effects of behavioural synchronizers are weaker in humans.

Finally, our analysis indicates that the apparent difference in the magnitude of phase advances and phase delays can be attributed in part to the longer than 24 h intrinsic period of the human circadian system (Czeisler *et al.* 1989, 1995*a*; Campbell *et al.* 1993). The slight deviation from 24 h of intrinsic period under these laboratory conditions would be expected to accumulate each day, resulting in a 2 h difference over the course of the 10 days between the initial and final phase assessments. This differential effect of intrinsic circadian period on the size of phase advance and phase delay shifts may explain in part why transmeridian travellers find it easier to adjust to westward travel (delay shifts) than to eastward travel (advance shifts) (Winget, DeRoshia, Markley & Holley, 1984).

The present study demonstrates the powerful impact of bright light on the human circadian timing system, and shows that sedentary activity, sleep–wake, social contact and food intake schedules have, at best, a much weaker resetting effect on the human circadian pacemaker than does light. In fact, the social contacts in the present study, while weaker than would be expected from individuals living in their home environment, were stronger than in the two classic studies used to justify social cues as the dominant entraining signal for human circadian rhythms (Wever, 1970; Aschoff *et al.* 1971). Therefore, we believe our present results are inconsistent with the hypothesis (Wever, 1970; Aschoff *et al.* 1971) that entrainment of the human circadian system occurs primarily by a mechanism (social entrainment) that is qualitatively different from the primarily photic entrainment mechanism found in nearly all other mammals. Our findings suggest that interventions employed to change the phase of the circadian system in humans, such as those which might be used to counteract the effects of jet-lag or to enhance adaptation to night shift work, should not rely solely on making changes to the timing of rest, sedentary activity and/or meal timing, unless such interventions concomitantly change the timing of exposure to the light–dark cycle. The present studies reaffirm the powerful effect of the light–dark cycle on circadian rhythms in humans.

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